ORGANIC LETTERS

2009
Vol. 11, No. 13
2719−2721

Reductive Phosphine-Mediated Ligation of Nitroxyl (HNO)

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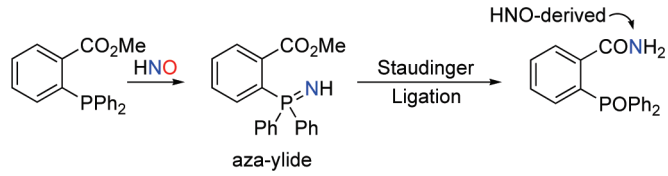
Received April 27, 2009

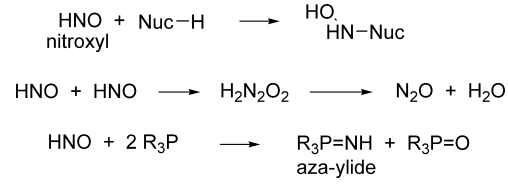
ABSTRACT



Nitroxyl (HNO) demonstrates a unique chemical and biological profile compared to nitric oxide (NO). Phosphorus NMR studies reveal that HNO reacts with triarylphosphines to give the corresponding phosphine oxide and aza-ylide. In the presence of a properly situated electrophilic ester, the aza-ylide undergoes a Staudinger ligation to yield an amide with the nitrogen atom being derived from HNO. These results define new HNO reactivity and provide the basis of new HNO detection methods.

Recent studies demonstrate the distinct biological and chemical properties of nitroxyl (HNO), the reduced/protonated form of the well-known signaling agent nitric oxide (NO).[1−3] Biologically, HNO increases cardiac contractility in both normal and failing canine hearts through mechanisms that include increased sarcoplasmic reticulum calcium release and uptake and increased calcium dependent force development in cardiac tissue.[4−7] These properties indicate the potential of HNO as a new therapy for congestive heart failure, a condition that causes more than 50 000 deaths each year in the United States.[8] Chemically, HNO represents the simplest nitroso compound (X−N═O, where X = H) and reacts as an electrophile with nucleophiles to yield addition products (Scheme 1).[2,3] Similar to C-nitroso compounds, nitroxyl dimerizes to form unstable hyponitrous acid, which dehydrates to nitrous oxide (N_2O, Scheme 1).[2,3] Nitroxyl dimerization complicates the study of its biology and chemistry as it requires: 1) HNO generation from donor molecules and 2) HNO detection through trapping with metal complexes, thiols or itself to form N_2O.[2,3] The previously reported reactions of organic phosphines with C and S-nitroso compounds to form phosphine oxides and aza-ylides suggest a similar reactivity with HNO (if viewed as a simplified nitroso compound).[9−12] Here we report the first reaction of

Scheme 1. HNO Reactions



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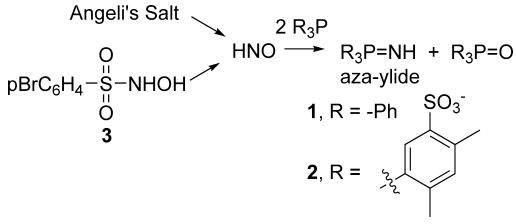
10.1021/ol900914s CCC: $40.75
Published on Web 06/03/2009

HNO with organic phosphines to produce the corresponding phosphine oxide and aza-ylide (Scheme 1). We show that in the presence of an electrophilic trap this aza-ylide intermediate reacts further to produce a stable and unique HNO-derived amide product (Scheme 4).

^{31}Phosphorus NMR experiments using authentic synthetic standards provide evidence for aza-ylide formation from the reaction of HNO with an organic phosphine. Mixture of Angeli's salt ($Na_2N_2O_3$), the most common HNO donor ($t_{1/2}$ = 2.8 min, 37 °C, pH 8.13−4.40),[13] with triphenylphosphine in dioxane/water (2:1) produces a mixture of triphenylphosphine oxide (δ = 31 ppm) and the triphenylphosphine-derived aza-ylide (**1**, δ = 26 ppm) as judged by ^{31}P NMR spectroscopy (Scheme 2 and

Scheme 2. Reaction of HNO and Triarylphosphines



Supporting Information). Liquid chromatography/mass spectrometry further confirms the identity of these products. Under these conditions aza-ylide (**1**), an intermediate in the Staudinger reduction of azides to amines[14] hydrolyzes to triphenylphosphine oxide as expected. ^{31}P NMR experiments show the formation of **1** and triphenylphosphine oxide, the expected byproduct of the reaction, within 5 min and the formation of no other phosphorus containing products. Control experiments show that triphenylphosphine does not directly react with Angeli's salt or nitrite, a byproduct of Angeli's salt decomposition to HNO.

^{31}P NMR experiments also reveal the reaction of Angeli's salt with the water-soluble phosphine tris(4,6-dimethyl-3-sulfonatophenyl)phosphine trisodium salt hydrate (TXPTS) in Tris buffer (100 mM, pH 7.6) produces two new phosphorus containing products in a 1:1 ratio (Figure 1). Comparison to authentic standards confirms the formation of the same aza-ylide derived from TXPTS (**2**, Scheme 2) and the corresponding phosphine oxide. Under these conditions, aza-ylide (**2**) demonstrates relative stability compared to **1**. ^{31}P NMR experiments indicate the presence of **2** along with increasing amounts of TXPTS phosphine oxide in this reaction mixture even after 6 days. Structural differences in the aromatic portion of the phosphines, especially the electron donating o and p methyl groups, likely influences the hydrolytic stability of aza-ylides **1** and **2**. TXPTS quenches

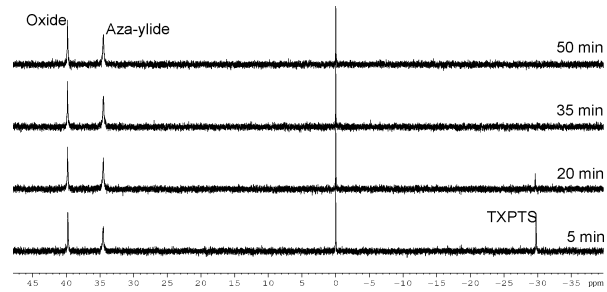


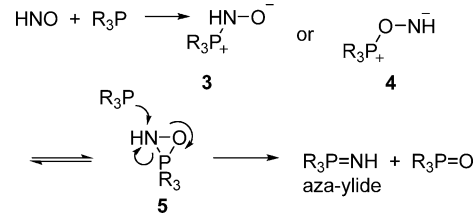
Figure 1. ^{31}P NMR spectrum of the reaction of Angeli's salt (0.04 mmol) with TXPTS (0.04 mmol) in 1:5 D_2O/Tris buffer over time. Resonances correspond to phosphine (−29.7 ppm), aza-ylide 2 (34.5 ppm), and phosphine oxide (39.8 ppm) and are referenced to 85% H_3PO_4 (0.0 ppm).

nitrous oxide formation (>90%) during the aqueous decomposition of Angeli's salt indicating reactivity with HNO. Mixture of TXPTS and Angeli's salt in buffer in the presence of glutathione (1 equiv) also forms aza-ylide (**1**) and the phosphine oxide of TXPTS suggesting that TXPTS reacts with HNO faster than glutathione.

Furthermore, the generation of HNO from 4-bromo-*N*-hydroxybenzenesulfonamide (**3**) under basic conditions in the presence of triphenylphosphine yields **1** and triphenylphosphine oxide (Scheme 2).[15] Using ^{15}N-labeled 4-bromo-*N*-hydroxybenzenesulfonamide results in ^{15}N labeled-**1** confirming the source of the nitrogen atom ($^1J_{N-P}$ = 31 Hz).[16] Under these conditions, **1** also hydrolyzes to triphenylphosphine oxide and control experiments indicate that triphenylphosphine does not directly react with **3**. In general, these experiments show that HNO, regardless of the source, reacts with organic phosphines to yield an aza-ylide.

The previously described reaction of *S*-nitrosothiols (another group of nitroso compounds, X−N═O, where X = −SR) with triarylphosphines to give the phosphine oxide and an aza-ylide provides insight to a possible mechanism for aza-ylide formation in this process.[10,11] Reaction of the phosphine with HNO could yield a product either through P-addition at N (**4**) or oxygen (**5**, Scheme 3). Each of these

Scheme 3. Proposed Mechanism of Aza-Ylide Formation



initial addition products could exist as a three-membered ring species (**6**, Scheme 3). Addition of a second phosphine to **6** (or to **4** or **5**) would give the corresponding aza-ylide and phosphine oxide in equal proportions.

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The strong evidence of aza-ylide formation provided by the preceding NMR studies suggests that a phosphine capable of undergoing the widely used Staudinger ligation should effectively provide a means for trapping HNO as the corresponding amide.[17] Treatment of the methyl ester (**7**) with Angeli's salt in a mixture of acetonitrile/Tris buffer (100 mM, pH = 7.0) at room temperature gives a 1:1 mixture of the benzamide phosphine oxide (**8**, δ = 34.4 ppm) and methyl ester phosphine oxide (**9**, δ = 33.3 ppm, Figure 2,

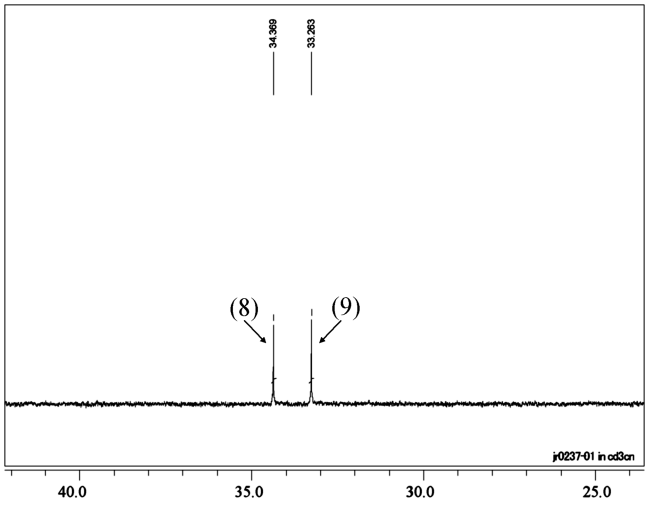
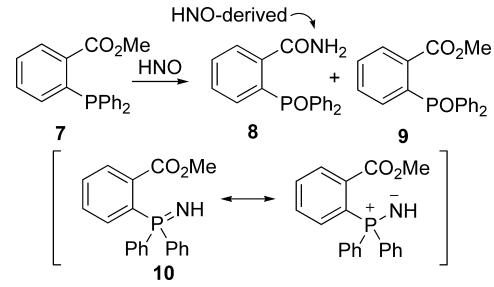


Figure 2. ^{31}P NMR spectrum of the reaction of Angeli's salt (0.03 mmol) with **7** (0.02 mmol) in 3:1 CD_3CN/Tris buffer at 3 h. Resonances correspond to **8** (34.4 ppm) and **9** (33.3 ppm) and are referenced to 85% H_3PO_4 (0.0 ppm).

Scheme 4) as judged by ^{31}P NMR spectroscopy. Liquid chromatography/mass spectrometry further confirms the identity of these products. Incubation of **7** with Angeli's salt on a preparative scale results in recovery of **8** in 60% yield (based on the theoretical amount of **8** formed in a 1:1 ratio from starting material) following chromatography. ^{31}P NMR experiments show the reaction to be complete within 20 min with the formation of no other phosphorus containing products (including the aza-ylide, Figure 2). Control experiments also indicate that **7** does not directly react with Angeli's salt or nitrite. Reaction of **7** with ^{15}N-**3** produces ^{15}N-**7** clearly demonstrating the amide nitrogen derives from **3** and presumably HNO. These results suggest the initial formation of aza-ylide (**10**, Scheme 4) by the reaction of HNO and **7** through a similar mechanism as Scheme 3. Intramolecular reaction of the aza-ylide group of **10** with the adjacent ester group generates the amide (**8**, Scheme 4).

These studies demonstrate that HNO reacts rapidly with organic phosphines to give the corresponding aza-ylide and

Scheme 4. Phosphorus Mediated Nitroxyl Ligation



phosphine oxide. These products differ from the reaction of triphenylphosphine with nitric oxide, which yields triphenylphosphine oxide and nitrous oxide.[19,18] The inherent reactivity of the aza-ylide allows ligation to a modified phosphine reagent producing a stable HNO-unique amide product. Develoment of these compounds as new HNO detection methods will require further investigation of their differential reactivity with NO and selective reaction compared to other biomolecules. The lack of specific HNO detection methods has impeded the understanding of HNO in biological systems. In summary, these experiments document the reactivity of HNO with triarylphosphines and provide insight into a new method for distinguishing this important biologically active nitrogen oxide.

Acknowledgment. This work was supported by the National Institutes of Health (HL 62198 and R21 087891). The NMR spectrometers used in this work were purchased with partial support from NSF (CHE-9708077) and the North Carolina Biotechnology Center (9703-IDG-1007).

Supporting Information Available: Experimental details for the synthesis and characterization, including ^{1}H, ^{13}C and ^{31}P NMR spectra, of compounds **1**−**3** and **7**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL900914S

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